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The H^+/K^+-ATPase inhibitory activities of Trametenolic acid B from *Trametes lactinea* (Berk.) Pat, and its effects on gastric cancer cells

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ABSTRACT

Trametenolic acid B (TAB), the bioactive component in the *Trametes lactinea* (Berk.) Pat, was reported to possess cytotoxic activities and thrombin inhibiting effects. This study was performed to investigate the effects of TAB on H^+/K^+-ATPase and gastric cancer. The H^+/K^+-ATPase inhibitory activity was determined by gastric parietal cells. Compared to the normal control group, TAB (10, 20, 40 and 80 μg/mL) inhibited the H^+/K^+-ATPase activity by 15.97, 16.96, 24.86 and 16.25%, respectively. In the study, 36 Kunming mice were randomly divided into six groups: control, model, TAB-L (TAB, 5 mg/kg/day, *i.g.*), TAB-M (TAB, 20 mg/kg/day, *i.g.*), TAB-H (TAB, 40 mg/kg/day, *i.g.*) and omeprazole (OL, 10 mg/kg/day, *i.g.*). All mice except the control group were administrated with anhydrous alcohol (5.0 mL/kg, *i.g.*) for induced gastric-ulcer 1 h after the 5th day. At the same time, the control mice were given the same volume of physiological saline. After 4 h, TAB was evaluated for H^+/K^+-ATPase inhibitory activities of ulcerative gaster, gastric ulcer index and ulcer inhibition. In vitro, the anti-proliferation effect of TAB to gastric cancer cell (HGC-27) in acid environment was detected by MTT, and the apoptosis morphological changes were also observed by Hoechst 33258 dye assay. The results indicated that TAB inhibited moderately H^+/K^+-ATPase activity in vitro. Compared to the model group, TAB showed anti-ulcer effects in gastric tissue with the dosages of 20 and 5 mg/kg in vivo. Apart from that, TAB could selectively inhibit gastric cancer cell viability and reduce cell apoptosis against HGC-27 cells at low doses in acid environment.

1. Introduction

Acid-related disorders are highly prevalent in the developed and cause heavy burden on health care systems [1]. Gastric acid

gastroesophageal reflux disease [2,3]. The gastric H^+/K^+-ATPase is the proton pump responsible for the final step of acid secretion in the stomach, which locates in gastric membrane vesicles and catalyzes the electro-neutral exchange of intracellular H^+ and extracellular K^+ coupled with the hydrolysis of cytoplasmic ATP [4,5]. Drugs for treatment of acid-related diseases are ultimately implemented through inhibiting H^+/K^+-ATPase activity [3,6,7]. A variety of heterocyclic structures has been described in

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literature as the gastric proton pump inhibitors (PPIs), for example omeprazole, esomeprazole, lansoprazole and pantoprazole, bind to the H^+/K^+-ATPase and have been available as therapeutics for a long time [8,9]. However, the adverse effect of long-term use of these drugs caused strong desire for investigators to develop new alternative medicine [10].

Peptic ulcer mainly occurs in the stomach and duodenal ampulla, which is a common digestive disease with increasing incidence year by year [11]. Gastric ulcer and duodenal ulcer are the common ulcers, and young males are easy to infect these diseases. It is apt to cause gastric cancer and other complications if not treated promptly [12]. There is a certain correlation between gastric ulcer and gastric cancer [13,14], and gastric ulcer is generally not cancerous in the early 3–5 years of its occurrence, but the risk of gastric cancer and the correlation were significantly increased with the duration and extension of pathogenesis, which are consistent with the pathogenesis of gastric. It thoroughly indicates that untimely treatment and allowing the development of gastric ulcer without restriction will greatly increase the chance of ultimate cancer [15,16]. Therefore, it is of great importance to enhance early prevention and treatment of gastric ulcer. Lots of traditional Chinese foods were reported to have positive effect on the gastric ulcer, and the active ingredients and anti-gastric ulcer mechanisms were worthy to be investigated.

Triterpenoids are kinds of economically and medicinally important natural products with wide uses for their attractive pharmacological medicinal activities [17]. The lanostane-type triterpenoid 3β-hydroxylanosta-8, 24-diene-21-oic acid (Trametenolic acid B, TAB) was the bioactive component of the *Trametes lactinea* (Berk.) Pat, isolated from China traditional Tujia food, fermented corn, which was reported to possess cytotoxic activities [18] and antimicrobial activity [19]. To the best of our knowledge, the anti-gastric ulcer and anti-gastric cancer effect of TAB was seldom investigated. In this work, the H^+/K^+-ATPase inhibitory activity, anti-proliferation effects against gastric cancer cells and computer-aided mechanism studies for TAB were firstly reported.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM) and L-glutamine were obtained from Gibco BRL (Grand Island, NY, U.S.A.). Fetal bovine serum (FBS) was purchased from ICN Biological, Inc. (Aurora, OH, U.S.A.) 0.25% Trypsin was purchased from Sino-American Biotechnology Company (Darmstadt, Germany). 3-(4, 5-Dimethyl-2-thiazolyl)-2,3-di-phenyl-2*H*-tetrazolium bromide (MTT), streptomycin and penicillin were purchased from Wako Pure Chemical Ind, Ltd (Osaka, Japan). Omeprazole was obtained from AstraZeneca. Trypan blue was purchased from Nanjing KeyGEN Biotech Co. Ltd. The H^+/K^+-ATPase ELISA kits were obtained from Nangjing Jiancheng Bioengineering Institute. All chemical reagents and materials were purchased from commercial suppliers and used without further purification. Column chromatography was performed using a 200–300 mesh silica gel. NMR spectra (DMSO-d_6) were recorded on Bruker Ultrashield™ 400 MHz Plus spectrometer. Elemental analyses were carried out with a Vario EL III elementary analysis instrument.

Male Kunming mice weighing 18–22 g were obtained from the Laboratory Animal Services Centre, China Three Gorges University, Yichang, China. The animals were maintained on a 12-h light/dark cycle under regulated temperature (22 ± 2 °C) and humidity (50 ± 10%), fed with standard diet and water ad libitum, and when necessary, animals were deprived from food allowing free access to water 12 h before the experiment. Experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals of China Three Gorges University, and approved by the ethics committee. The whole laboratory procedure was carried out under the permission and surveillance of the ethics committee.

TAB and OL were dissolved in DMSO at high concentrations. Once dissolved in the solvent, all compounds were stored at −20 °C. Compounds were diluted to relevant concentrations with medium or physiological saline when in vivo and in vitro experiments.

2.2. Experimental design

2.2.1. Extraction and isolation of TAB

T. lactinea was succeeded in culture in our laboratory, which was identified as *T. lactinea* (Berk.) Pat. by professor Shaobai Wang of Hubei Sanxia Science and Technology School. The mycelium of *T. lactinea* (120 g) was extracted with ethanol (95%) under 50 °C for 3 h. After the removal of solvent under reduced pressure, 63 g extract was obtained, which was extracted with ethyl acetate and gave 35 g residue [20–22]. Finally, the 3β-hydroxylanosta-8, 24-diene-21-oic acid (Trametenolic acid B, 7.0 g, Yield: 5.8%) was obtained with the purity of >98% by silica gel column chromatography (*n*-hexane/EtOAc = 4:1, *v*/*v*), which was characterized by ^{1}H NMR on Bruker Ultrashield™ 400 MHz Plus spectrometer, and the spectra were identical to literature reports (its structure was shown in Fig. 1). Its data of ^{13}C NMR and ^{1}H NMR spectra are as follows: ^{13}C NMR (DMSO-d_6, 500 MHz):177.1, 134.4, 133.5, 131.2, 124.0, 76.9, 50.2, 49.2, 47.6, 46.7, 43.9, 38.7, 36.7, 35.4, 32.3, 30.2, 28.5, 28.2, 27.7, 26.6, 26.1, 25.7, 25.6, 24.2, 20.4, 19.1, 18.0, 17.5, 15. 9, 15.7; ^{1}H NMR (DMSO-d_6, 500 MHz): 0.68 (s, 3H), 0.69 (s, 3H), 0.81 (s, 3H), 0.90 (s, 3H), 0.90 (s, 3H), 1.52 (s, 3H), 1.62 (s, 3H), 4.25 (d, J = 3.5 Hz, 1H), 5.06 (s, 1H), 11.96 (s, 1H).

2.2.2. Gastric parietal cells

Kunming mice (20 ± 2 g) were sacrificed by cervical dislocation. The abdomen was cut open, the stomach was perfused through the aorta with phosphate buffered saline (PBS, in mM: NaCl 149.6, K_2HPO_4 3, NaH_2PO_4 0.64, pH 7.4 at 37 °C) and excised [23]. The fundus and antral regions were discarded, and the mucosa was freed from the muscular layers, minced and digested in trypsin solution (0.25%) for 10 min at 37 °C. The gastric parietal cell suspension was filtered through a nylon mesh (750 nm), centrifuged at 1200 rpm for 7 min, rinsed four times with PBS containing 0.5% bovine serum albumin by sedimentation, and resuspended in PBS [24,25]. The viability of the isolated gastric parietal cells was greater than 90% as determined by trypan-blue dye exclusion [26].

2.2.3. Induction of acute gastric lesions in mice

36 Kunming mice were randomly divided into six groups: control, model, TAB-L (TAB, 5 mg/kg/day, *i.g.*), TAB-M (TAB, 20 mg/kg/day, *i.g.*), TAB-H (TAB, 40 mg/kg/day, *i.g.*) and

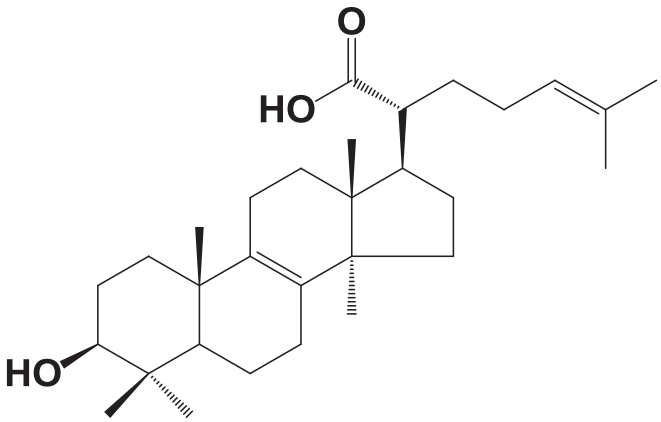

Fig. 1. The structure of Trametenolic acid B.

omeprazole (OL, 10 mg/kg/day, *i.g.*). All mice except the control group were administrated with anhydrous alcohol (5.0 mL/kg, *i.g.*) for gastric-ulcer induction 1 h after the 5th day. At the same time, control mice were given the same volume of physiological saline. On the 4th day of feeding, mice were in absolute diet for 12 h with free access to water before the experiment. On the 5th day, 1 h later after the last administration, all mice were administrated with anhydrous alcohol (5.0 mL/kg, *i.g.*) for gastric-ulcer induction. The animals were sacrificed by cervical dislocation after 4 h of alcohol induction. The stomachs were removed and cut along the greater curvature of the stomach, discarded the fundus and antral regions, and the glandular mucosa was quickly weighed, frozen down and stored at − 80 °C for further determinations of H^+/K^+-ATPase activity [19,27].

2.2.4. Determination of H^+/K^+-ATPase activity in vitro

Depurated gastric parietal cell suspension was seeded at 40–50 cells/well in a 96-well plate, in DMEM medium supplemented with 10% FBS, penicillin and streptomycin. The cells were incubated with various concentrations of TAB in a final volume of 200 μL medium for 2 h at 37 °C/5% CO_2. After that, H^+/K^+-ATPase activity was measured by the determination of H^+/K^+-ATPase ELISA kit according to its instruction. To define one enzyme activity unit is 1 μmol inorganic phosphorus produced from ATP breaking that by 1 mg of ATP enzymes per hour.

2.2.5. Determination of H^+/K^+-ATPase activity in vivo

The mucosa from experimental mice was made of a 10% homogenate in accordance with the proportion of gastric mucosal tissue weight with saline 1:9 and centrifuged at 2500 rpm for 10 min, and diluted with normal saline into a 2% homogenate [28–30]. H^+/K^+-ATPase activity was measured by the determination of H^+/K^+-ATPase ELISA kit according to its instruction.

2.2.6. Molecular docking

The sequence of the human gastric H^+/K^+-ATPase receptor (1035 amino acids) was taken from the Swiss-ProtDatabase (ID: P20648). Through blasting sequences from the Protein Data Bank [31], the crystal structure of sodium–potassium ATPase (PDB ID: 2ZXE) [32] was used as a template. The sequence alignment was carried out using the ClustalW algorithm [33]. The homology model of H^+/K^+-ATPase was generated using MODELLER9v3 [34]. The resultant structure of the H^+/K^+-ATPase receptor was subject to the Protein Preparation Wizard module in Schrödinger [35] as follows: adding hydrogen, assigning partial charges using the OPLS-2001 force field, and assigning protonation states. Then the minimization was carried out using the OPLS force field in the MacroModel module in Schrödinger. The minimized structure was validated using PROCHECK [35,36]. The final optimized model of the gastric H^+/K^+-ATPase receptor was used to dock the ligand. The Glide (SP mode) protocol in the Schrödinger software suite [35] was carried out between TAB and H^+/K^+-ATPase. The final ligand–protein complex was visualized using PyMOL0.99 [37].

2.2.7. Cell culture and cell viability analysis

Human gastric cancer HGC-27 cells and human gastric mucosa epithelial GES-1 cells were obtained from The Cell Bank of Type Culture Collection of Chinese Academy of Sciences, Shanghai Institute of Cell Biology, Chinese Academy of Sciences, which were cultured in DMEM medium supplemented with 10% FBS and 100 units/mL penicillin in a humidified 5% CO_2 atmosphere. Cell viability was assessed by dye exclusion assay. To evaluate the effect of TAB on cell viability, gastric cancer HGC-27 cells and normal human gastric epithelial GES-1 cells were exposed to TAB with different concentrations (10, 5 and 1 μg/mL), and at the same time, HGC-27 cells were treated with drugs in different pH medium including pH 7.5, 6.5, and 5.5 for 24 h, and set omeprazole (10 μg/mL) as the positive control. The cells were harvested and stained with Trypan blue. Survival (%) was calculated by the following formula: No. of viable cells (dye excluded cells) / No. of total [38].

2.2.8. Assessment of cell morphological changes

The examination of morphological changes was conducted by Hoechst 33258 staining of nuclei under the instruction of the apoptosis-Hoechstwas visualized with a fluorescence microscope.

2.2.9. Statistical analysis

Each experiment was repeated at least three times. Data were processed with the SPSS 13.0 for Windows software package. Values were expressed as mean ± standard

deviations (SD). One-way analysis of variance was used for statistical analysis. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Decrease H^+/K^+-ATPase activity in vitro

The in vitro H^+/K^+-ATPase inhibitory activity of TAB was determined by the determination of H^+/K^+-ATPase ELISA kit. The results were listed in Fig. 2, and the H^+/K^+-ATPase inhibitory activity of omeprazole significantly decreased showing percentage protection of 63.7% compared to the control group. Concomitantly, it was observed that the activity of H^+/K^+-ATPase was decreased by TAB (5, 10, 20 and 40 μg/mL) by 4.72, 15.97, 16.96 and 24.86%, respectively. However, its inhibition ratio decreased nearly 16.25% at the dose of 80 μg/mL. Graded doses of TAB at a certain range prevented the activity of H^+/K^+-ATPase moderately that presented dose-dependent, and the prevention of the activity of H^+/K^+-ATPase at the high dose (80 μg/mL) that over this range was more minor.

3.2. Inhibit H^+/K^+-ATPase activity in vivo

As shown in Fig. 3, the H^+/K^+-ATPase activity in anhydrous ethanol-induced mice gaster (ulcer control value = 9.461 ± 1.585 μmol Pi/mg prot/h, mean ± SD) was much higher than that of the control group (normal control value = 5.229 ± 0.460 μmol Pi/mg prot/h). Furthermore, intragastric administration treatment with the standard drug, omeprazole (OL, 10 mg/kg, *i.g.*) and graded doses of TAB (5 and 20 mg/kg, *i.g.*) significantly reduced the H^+/K^+-ATPase activity in anhydrous ethanol-induced mice in 49, 37 and 29%, respectively in comparison with the model group. And the H^+/K^+-ATPase activity in the 40 mg/kg dose group (high dose group value = 6.982 ± 0.377 μmol Pi/mg prot/h) was decreased in 17%, but the difference was not statistically significant compared with the control group ($P > 0.05$).

3.3. Molecular docking

The sequence identity between sodium–potassium ATPase (PDB ID: 2ZXE) and human gastric H^+/K^+-ATPase (Swiss-Prot ID: P20648) was 64%. The PROCHECK G-factor ranked values above −0.5 as positive candidates for homology models. Based on the PROCHECK verification, the G-factor value of the H^+/K^+-ATPase model generated by MODELLER was −0.22 (exceeding −0.5). Therefore the model could be regarded as structurally realistic. The final gastric H^+/K^+-ATPase homology model is shown in Fig. 4.

Docking simulation between TAB and H^+/K^+-ATPase was performed. The Glide GScore was −4.75. The docking results were shown in Fig. 5. The hydroxyl and carboxyl groups of TAB formed hydrogen bonds with the amino acid residues Tyr804 (distance: 2.21 and 2.29 Å), Gln926 (2.31 Å), and Ile816 (1.82 Å).

3.4. Effect of TAB on cell proliferation in an acidic microenvironment

To assess the selective effect of TAB on HGC-27 cell, we studied responses of these cells to treatments by TAB in normal pH condition (pH = 7.5). Cells were exposed to TAB over the concentration range of 1–80 μg/mL. The IC_{50} values of TAB on HGC-27 and GES-1 cells were 15.91 and 35.99 μg/mL and the maximum non-toxic concentrations (inhibition rates ≤ 10%) of TAB on these cell lines were 4.07 and 8.81 μg/mL, respectively. We chose 10 μg/mL as the maximum experimental concentration, which had nearly no effect on normal gastric parietal cells. We tested the effects of TAB in different concentrations on cell growth in culture media maintained at various pHs. As shown in Fig. 6, TAB significantly attenuated viability of HGC-27 cell in a dose- and pH-dependent manner. At pH 5.5, TAB (1, 5 and 10 μg/mL) induced cell death proportions to 48.2, 61.4 and 81.5%, respectively. However, TAB showed a slight effect on normal gastric epithelial cells (GES-1) at these doses. There was more than 80% viability at the dose of 10 μg/mL for GES-1 cells in Fig. 7.

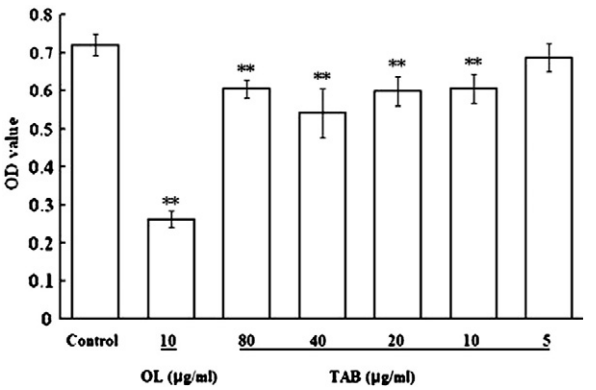

Fig. 2. Inhibition of H^+/K^+-ATPase by different concentrations of TAB in vitro. Data are shown as the mean ± SD (n = 6). *P < 0.05, **P < 0.01 compared with control group.

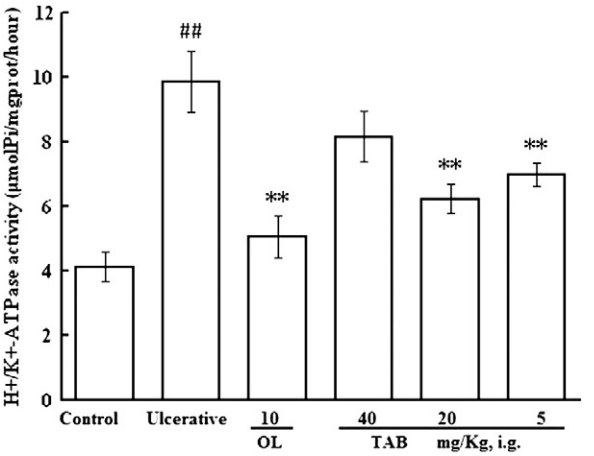

Fig. 3. Effect of TAB on decreasing H^+/K^+-ATPase in comparison to omeprazole in anhydrous ethanol-induced gastric ulcer model. Data are shown as the mean ± SD (n = 6). #P < 0.05, ##P < 0.01 compared with control group; *P < 0.05, **P < 0.01 compared with model group.

Fig. 4. The homology model of gastric H^+/K^+-ATPase.

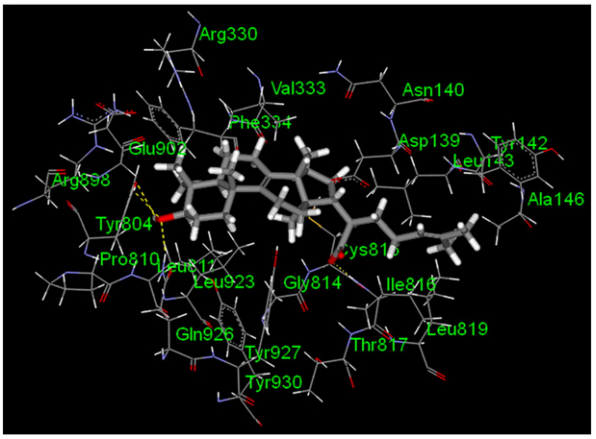

Fig. 5. The docking results of TAB with H^+/K^+-ATPase.

3.5. Effect of TAB on cell apoptosis in HGC-27 cells

To determine whether the proliferation-inhibitory effect of TAB was related to the induction of apoptosis, morphological assay of cell death was investigated by a phase contrast microscope. Marked morphological changes could be seen after 24 h with characterization of shrinkage of cells and loss of the originally confluent monolayer (Fig. 8B, E, F, G and H), indicating that apoptotic cell death was induced by treatment of omeprazole and TAB. To further examine the morphological changes in responding to TAB treatment, both control and TAB treated cells were stained with the fluorescent dye Hoechst 33258 and visualized by a fluorescent microscope. The control cells were normal and the nuclei were round and homogeneous (Fig. 8A), while the cells treated with clitocine after 24 h exhibited the typical characteristics of apoptosis. In the present study, TAB (Fig. 1) was isolated from *T. lactinea* (Berk.) Pat and characterized via NMR and ESIMS, which could inhibit the H^+/K^+-ATPase activity both in vitro and in vivo. The possible anti-ulcer mechanism of TAB was also analyzed through computer-aided docking simulation. In vitro, TAB inhibited H^+/K^+-ATPase activity at the concentration range of 10–40 μg/mL in a dose-dependent manner. On the other side, TAB also showed moderate anti-ulcer effects compared with the omeprazole group in vivo.

4. Discussion

Our present study indicated that TAB possessed moderate H^+/K^+-ATPase inhibitory activity in vitro. Compared to the model group, TAB showed anti-ulcer effects in gastric tissue with the dosages of 20 and 5 mg/kg in vivo. Apart from that, TAB could selectively inhibit gastric cancer cell viability and induce cell apoptosis at low doses in HGC-27 cells in acid environment.

Most prevalent gastrointestinal disorders and acid-related diseases such as peptic ulcer are produced by gastric acid. The gastric H^+/K^+-ATPase is the proton pump responsible for the final step of acid secretion in the stomach, which is an important gastric acid regulator in gastric membrane vesicles. The major approach in the development of drugs against these acid-related diseases is still by inhibiting the gastric H^+/K^+-ATPase activity. Nevertheless, the adverse effect of long-term use of these drugs is one of the major limitations in clinical applications [7,13]. Furthermore, gastric ulcer and duodenal ulcer are sometimes apt to cause gastric cancer and other complications if not treated promptly. Our results indicated that graded doses of TAB possessed moderate H^+/K^+-ATPase inhibitory activity in vitro. Compared with the model group, TAB (5 and 20 mg/kg, *i.g.*) could also reduce the H^+/K^+-ATPase activity in vivo on ethanol-

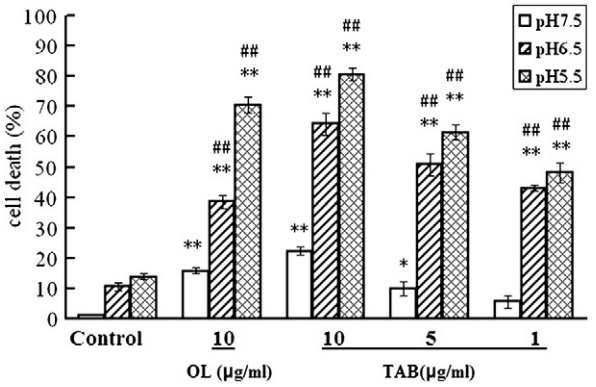

Fig. 6. Reduction of cell viability by treatment of TAB in culture media maintained at various pHs. HGC-27 cells were treated with 1, 5 and 10 μg/mL of TAB for 24 h. Data are shown as the mean ± SD (n = 3). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with control group; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with different pH groups.

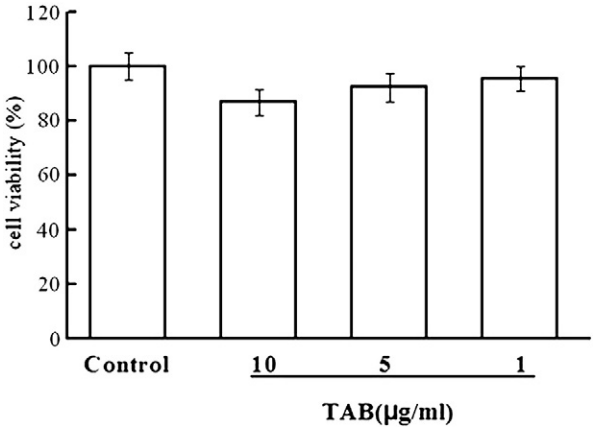

Fig. 7. Survival rate of GES-1 cells treated by TAB with 1, 5and 10 μg/mL for 24 h. Data are shown as the mean ± SD (n = 3). $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with control group.

induced mice with the inhibitions of 29% and 37%, respectively. At the dose of 40 mg/kg, the inhibitory effect of TAB was inferior to the doses of 20 mg/kg and 5 mg/kg. These results were consistent with the results in vitro, which indicated that the inhibition on H^+/K^+-ATPase activity was notable in certain doses (10–40 μg/mL in vitro, 5 and 20 mg/kg in vivo), but the inhibition of high dose (80 μg/mL in vitro and 40 mg/kg in vivo) was reduced rather than enhanced. Furthermore, the computer-aided molecular docking simulations also indicated that TAB had good binding abilities to the H^+/K^+-ATPase. Therefore, all of these results proved that TAB showed the potential drug safety and druggability because of its widely biological activity and low-toxicity.

Proton pump inhibitors were widely reported to selectively inhibit cell proliferation of gastric cancer [23,39,40], induced gastric cancer apoptosis in a time- and dose-dependent manner under acidic environment and had no effect on normal gastric epithelial cells [41]. For example, pantoprazole could induce apoptosis of gastric cancer cells selectively, significantly decrease tumor volume and induce tumor cell apoptosis in large scale after intratumoral injection in vivo [42,43]. Our present findings showed that low doses of TAB were able to reduce gastric cancer cell viability and induce cell apoptosis in acidic environment at a pH- and dose-dependent manner. On the other side, TAB nearly has no effect on normal gastric epithelial cells at these

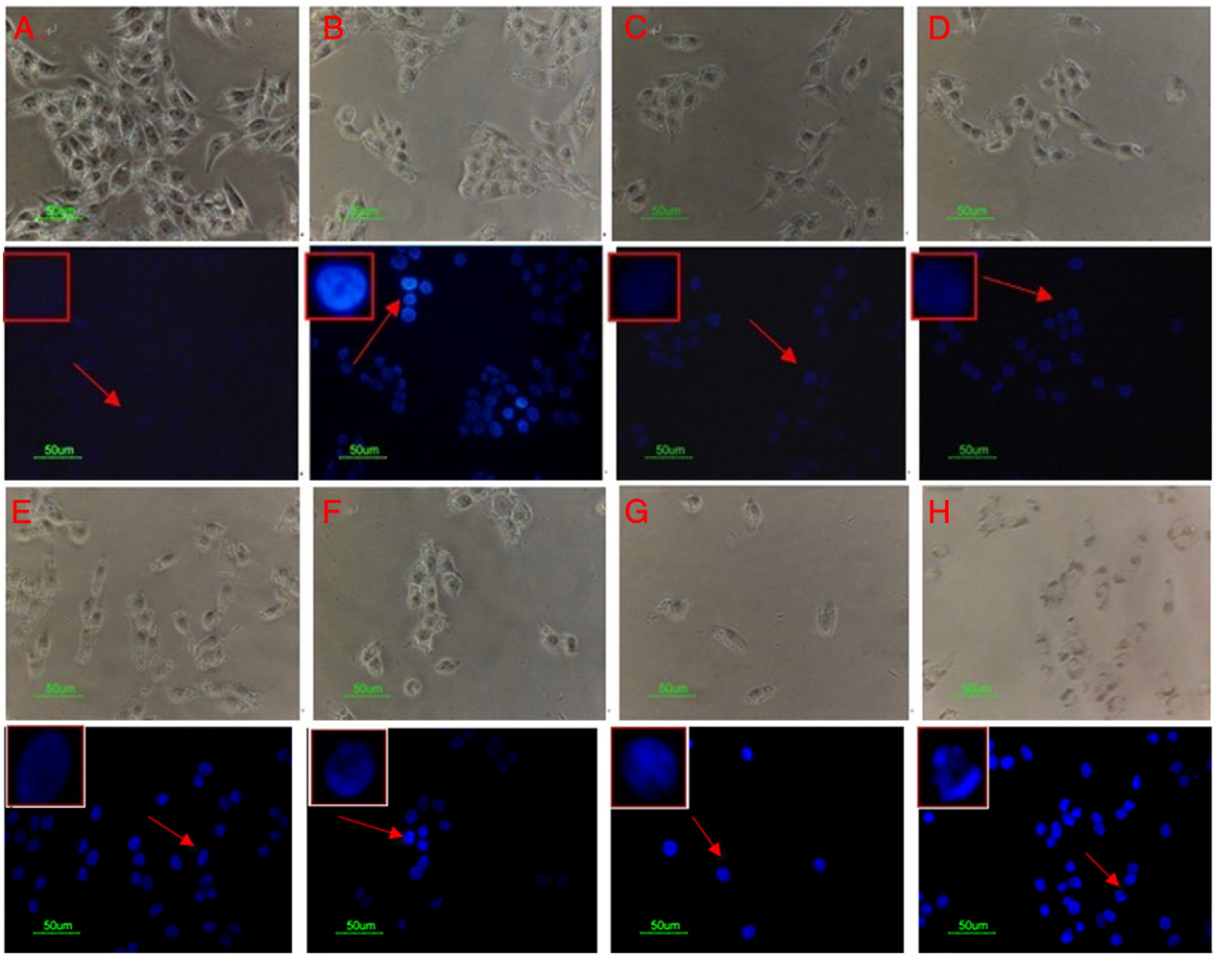

Fig. 8. Morphological changes of HGC-27 cells after treatment or untreated with omeprazole (10 μg/mL) and TAB (1, 5, 10, 20, 40 and 80 μg/mL) for 24 h. Every group was observed by phase-contrast and fluorescence microscope. A, B, C, D, E, F, G and H represented untreated cells group, positive group (omeprazole, 10 μg/mL) and TAB treated cells group (1, 5, 10, 20, 40 and 80 μg/mL) in order.

circumstances. Since TAB showed a high selectivity on cell proliferation inhibition to gastric cancer cell line HGC-27, and has considerable cell apoptosis induction on HGC-27, we conjectured that its anti-gastric cancer mechanism might be related to the reduction on gastric acid secretion through inhibiting the activity of H^+/K^+-ATPase. TAB could induce apoptosis in a concentration-dependent manner in HGC-27cell and had slight effect on normal gastric epithelial cells. This provides a new approach to the treatment of gastric cancer and protection of normal gastric epithelial cells.

Literatures report that TAB possesses wide bioactivities including tumor cell anti-proliferation effects (for example, human HL-60 leukemia, human KB epidermoid carcinoma, murine L1210 leukemia cells, Caski, HT-3, and T-24) and enzyme inhibitory activity (human thrombin, bovine trypsin and so on) [18,44]. In our previous preliminary experiment results, TAB also found to possess anti-proliferative effects against other cancer cell lines including hepatocellular carcinoma HepG2, lung cancer A549, breast cancer MDA-MB-231, MCF-7 and gastric cancer cell HGC-27 for 48 h with the IC_{50} values of 27.14, 43.42, 32.30, 19.44 and 15.91 μg/mL, respectively. In these anti-tumor activities data, TAB seemed to be more sensitive to gastric cancer cell HGC-27, which significantly inhibited HGC-27 cell proliferation in acidic environment with the IC_{50} values of 1.54 and 4.19 μg/mL for 24 h at pH 5.5 and pH 6.5. The anti-tumor mechanism of TAB might be related to the H^+/K^+-ATPase inhibitory activity by blocking the ATP-sensitive K^+ channel and resulting in the membrane depolarization, opening the voltage sensitive Ca^{2+} channel and increasing the intracellular concentration of Ca^{2+}, selectively induce apoptosis of gastric cancer HGC-27 cells, but the exact mechanism required further study.

In this study, our work demonstrates that TAB could inhibit the H^+/K^+-ATPase activity both in vitro and in vivo, which was confirmed by the computer-aided docking simulations. In acidic environment, lower doses of TAB also exhibited significantly anti-proliferative effect against gastric cancer HGC-27 cells with apoptosis-inducing mechanisms in a dose-dependent manner. The results of this study indicated that the anti-gastric cancer effects of TAB might be related to the H^+/K^+-ATPase inhibitory effects.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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